Australian Journal of Crop Science AJCS
AJCS 9(1):1-8 (2015) ISSN:1835-2707

Involvement of ethylene in causation of creasing in sweet orange [*Citrus sinensis* (L.) Osbeck] fruit

Zahoor Hussain, Zora Singh*

Curtin Horticulture Research Laboratory, Department of Environment and Agriculture, School of Sciences, Faculty of Science and Engineering, Curtin University, GPO Box U 1987, Perth 6845, Western Australia, Australia

***Corresponding author: Z.Singh@curtin.edu.au (Zora Singh)**

Abstract

Creasing is a physiological disorder in the rind of sweet orange [*Citrus sinensis* (L.) Osbeck] fruit and causes serious economic losses. The involvement of ethylene in creasing of sweet orange fruit was investigated by monitoring the comparative changes in endogenous levels of ethylene in the creased and normal fruit. The effects of exogenous applications of Ethrel® (2-chloroethylphosphonic acid) and inhibitors of ethylene biosynthesis on endogenous levels of ethylene in the fruit and creasing index (CI) were also examined. Endogenous levels of ethylene in the normal and the creased fruit of Navelina, Washington Navel, Lane Late and Valencia Late sweet orange were determined at harvest maturity. The effects of different concentrations of Ethrel® applied at fruit maturity (165 days after fruit set) on endogenous levels of ethylene in the fruit were determined 30 to120 days after spray (DAS) and CI at fruit harvest. The efficacy of different concentrations of ethylene inhibitors such as aminoethoxyvinylglycine (AVG) or CoSO4 were sprayed at the golf ball stage (fruit diameter 40 ± 5 mm) on CI at harvest was also tested. Endogenous levels of ethylene were significantly higher in the creased fruit than the normal ones in different cultivars of sweet orange including Navelina, Washington Navel, Lane Late and Valencia Late. Exogenous spray application of ethrel at the rate of 250 -750 mg L^{-1} on mature fruit elevated the endogenous levels of ethylene in the fruit 30 to120 DAS and increased CI as compared to the control in Washington Navel and Lane Late. Whilst, the spray application of ethylene inhibitor AVG (20-60 mg L^{-1}) or cobalt sulphate ($CoSO_4$) (125-500 mg L^{-1}) at the golf ball stage was more effective in reducing CI compared to the control in all cultivars during 2011 and 2012. A spray application of AVG (60 mg L^{-1}) at the golf ball stage was more effective in reducing CI (16.14 % and 15.93%) compared to the control (34.18 and 35.25 %) in cv. Washington Navel during 2011 and 2012, respectively. Similarly, the application of $CoSO_4$ (500 mg L^{-1}) at the golf ball stage was more effective in reducing CI (19.32% and 19.32 %) compared to the control (32.11 and 38.46 %) in cv. Navelina during 2011 and Lane Late during 2012, respectively. In conclusion, higher levels of endogenous ethylene in the creased fruit and promotion of the CI with the exogenous application of ethrel and its reduction with the application of ethylene inhibitors suggested the involvement of ethylene in the causation of creasing in sweet orange fruit.

Keywords: Ethylene; Sweet Orange; Creasing Index; Inhibitors of ethylene.
Abbreviations: CI%_creasing index percent; AVG_aminoethoxyvinylglycine; CoSO4_cobalt sulphate; DAS_days after spray; DAFS_ days after fruit set; ACS_1-aminocyclopro- pane-1-carboxylase synthase; ACC_1-aminocyclopropane-1-carboxylic acid; SAM_S-adenosylmethionine; *exo*-PG_ *exo*-polygalacturonase , *endo*-PG_ *endo*-polygalacturonase, PE_pectin esterase and EGase_*endo*-1,4-β-D-glucanase.

Introduction

Creasing (albedo breakdown) is a physiological disorder in the rind of sweet orange fruit. The symptoms of creasing include multiple cracking in the pitted peel because of albedo decomposition (Li et al., 2009), separation of cells in the albedo tissue, resulting in channels in the fruit rind (Treeby et al., 1995). The creasing also creates a weak point in the flavedo, which cause cracks and fruit is ruptured following packaging for fresh markets (Gilfillan et al., 1981). Creasing has been reported in different orange-producing countries of the world, including Australia (Treeby et al., 1995), USA (Jones et al., 1976), South Africa (Holtzhausen, 1981), Spain (Agustí et al., 2003) and China (Li et al., 2009). Although, the symptoms of creasing are visible at fruit maturity, losses due to creasing vary among seasons, location, and cultivars, sometimes exceed more than 50% (Gilfillan et al., 1981). Creasing has been reported to be associated with genotype (Agustí et al., 2003), climate (Jones et al., 1967; Gambetta et al., 2000), rootstock (Treeby et al., 1995), crop load (Jones et al., 1967), rind thickness (Holtzhausen, 1981), irrigation (Agustí et al., 2004) and mineral nutrition (Ali et al., 2000; Bower, 2004). Numerous attempts have been made to control the physiological disorder, but none appear to have conclusively identified the physiological changes leading to this disorder (Bower, 2004). It is well known that boron is a structural element of the plant cell wall and boron spray in early summer is effective in reducing creasing in sweet orange (Pham, 2009). In Australia, calcium spray has been used to control creasing, but coupled with a limited reduction in creasing (25 to 30 %) (Treeby et al., 2000; Pham et al., 2012). Similarly, gibberellic acid has been used to control creasing in South Africa; however, it does not prevent creasing but only delays the onset of creasing (Bower, 2000). Ethylene acts as an inducer for fruit ripening (Bleecker, 2000). Ethylene is a key signal compound involved directly

in regulation of fruit ripening, senescence and fruit quality (Manjunatha et al., 2012). Ethylene is very effective at lower concentrations ranging from a part-per-million (µl l^{-1}) to part-per-billion (nl l^{-1}) in regulating ripening, senescence in many fruit, vegetables and ornamentals (Saltveit, 1999). Ethylene accelerates softening in citrus fruit due to disintegrating cell membranes to make them leakier (Rath and Prentice, 2004; Ladaniya, 2007). The exogenous application of ethylene promotes respiration rate, and ripening as well as improves colour development in citrus (Ladaniya, 2007; Burg, 2004; Agustí et al., 2002). But the pre-harvest application of ethrel does not affect fruit quality parameters such as soluble solids concentration, juice percentage, fruit weight, rind thickness, acidity and ascorbic acid in the citrus (Al-Mughrabi et al., 1989). As a prelude, the visible symptoms of creasing on sweet orange fruit appears mainly after fruit maturation and coupled with ripening and/over-ripening process, which also involve cell separation (Monselise et al., 1976; Saleem et al., 2014). A substantial increase in the incidence of creasing with delayed harvesting in Washington Navel sweet orange fruit suggest that the creasing is coupled with the ripening and/ over-ripening processes (unpublished data). Citrus is classified as a non-climacteric fruit, but produce only small amount of ethylene, while ethylene plays an important role in changing fruit colour, flavour, chemical composition and texture in citrus fruit (Aharoni, 1968; Ekas, 1970; Ladaniya, 2007). The development of creasing in sweet oranges has been associated with increased water soluble pectins consequently leading to earlier senescence of albedo tissue (Monselise et al., 1976). Recently, Saleem et al. (2014) claimed that higher activities of pectinesterase (PE), *exo*-polygalacturonase (*exo*-PG), endo- polygalacturonase (*endo*-PG), and endo-1, 4-*ß* -D-glucanase (EGase) in the albedo of creased fruit at commercial harvest seem to be associated with enhanced loss of pectins and starch in the cell walls of albedo tissue, leading to cell wall loosening and formation of cracks which consequently reduce hardness, stiffness and tensile force of the rind. Earlier, ethylene biosynthesis in the albedo tissue of 'Satsuma' mandarin fruit has been reported by Hyodo (1977). Some preliminary and sporadic reports suggested higher levels of endogenous ethylene in the albedo tissue of creased fruit than the normal ones in Valencia orange (Monselise et al., 1976). Similarly, Pham (2009) reported higher levels of endogenous ethylene in the rind of creased fruit than normal ones in Washington Navel sweet orange. However, the *in vivo* role of ethylene involving exogenous applications of ethylene and its inhibitors on creasing of sweet orange fruit is yet to be investigated. We hypothesised that ethylene plays a key role in modulating creasing in sweet orange fruit. We investigated the comparative changes in the concentrations of endogenous ethylene in the creased and normal fruit of four different cultivars of sweet orange. The effects of exogenous application of different concentrations of ethrel at fruit maturation stage, and ethylene inhibitors such as aminoethoxyvinylglycine (AVG) and cobalt sulphate ($CoSO_4$) at golf ball stage in regulating the incidence of the creasing in sweet oranges were also investigated.

Results

Endogenous levels of ethylene in normal and creased fruit of different cultivars of sweet orange

The creased fruit of Navelina, Washington Navel, Lane Late and Valencia Late sweet orange exhibited significantly ($p \leq 0.05$) higher levels of endogenous ethylene (1.29, 1.18, 1.16, 0.86 µL. $kg^{-1}.hour^{-1}$) than normal ones (1.06, 0.81, 0.98, 0.51 µL. $kg^{-1}.hour^{-1}$) respectively (Fig. 1). When averaged over cultivars, the creased fruit exhibited significantly higher mean endogenous level of ethylene (1.12 µL. $kg^{-1}.hour^{-1}$) than the normal ones (0.84 µL. $kg^{-1}.hour^{-1}$). Mean endogenous level of ethylene differed significantly ($p \leq 0.05$) among different cultivars of sweet orange. The interaction between type of fruits (creased and normal) and cultivars was found to be non-significant ($p \leq 0.05$) for endogenous levels of ethylene.

Effects of exogenous application of different concentrations of ethrel on endogenous level of ethylene in cv. Washington Navel and Lane Late fruit

All the ethrel treatments applied at165 days after fruit set (DAFS) significantly ($p \leq 0.05$) increased endogenous level of ethylene in the fruit at 30 DAS in Washington Navel and Lane Late sweet orange as compared to control (Table 1). When averaged over cultivars, the mean endogenous levels of ethylene 30 DAS in the fruit increased with the increased concentrations of ethrel applied but the differences among treatments were not significant. Whilst, 60, 90 and 120 DAS, all the ethrel treatments have significantly increased mean endogenous levels of ethylene in the fruit than untreated control, but the treatments of 750 mg L^{-1} ethrel resulted in highest levels of endogenous ethylene as compared to all other treatments and control. The effect of cultivars and interaction of cultivar with ethrel treatments were found to be non-significant ($p \leq 0.05$) for endogenous levels of ethylene in the fruit 60, 90 and 120 DAS.

Effect of exogenous application of different concentrations of ethrel on creasing index at mature stage in Washington Navel and Lane Late

All the ethrel treatments significantly ($p \leq 0.05$) increased mean CI as compared to the control and the creasing was more pronounced at higher concentration of ethrel applied. A similar trend of increased mean CI with exogenously applied ethrel was recorded in cv. Washington Navel and cv. Lane Late sweet oranges (Fig. 2). In general, the CI was higher in cv. Washington Navel (50.71%) than Lane Late (43.52%) at higher concentration of ethrel (750 mg. L^{-1}) applied. The effect of cultivars and interaction of cultivar with treatments were found to be significant ($p \leq 0.05$) for creasing index.

Effect of exogenous application of aminoethoxyvinylglycine (AVG) and $CoSO_4$ applied at golf ball stage on CI of sweet orange

All the treatments of AVG applied at a golf ball stage significantly ($p \leq 0.05$) reduced CI in sweet orange cv. Navelina, Washington Navel and Lane Late in 2011 and 2012 (Fig. 3). When average over cultivars, the spray treatment of AVG (60 mg L^{-1}) resulted in lowest mean CI (18.29 and 18.45 %) than the control (34.08 and 36.86%) and all other treatments in 2011 and 2012. The interaction between the treatments and cultivars were found to be non-significant ($p \leq 0.05$) for CI during 2011 and 2012. The CI was significantly ($p \leq 0.05$) reduced with the increased concentration of $CoSO_4$ (125 to 500 mg L^{-1}) applied at a golf ball stage compared to the control and all other treatments in 2011 and 2012 (Fig. 4). The foliar application of $CoSO_4$ (500 mg L^{-1}) resulted in significantly lower mean CI (21.80 and 19.79 %) than the control (34.08 and 36.86%) during 2011 and 2012, respectively. When average over treatments, the mean CI was

Table 1. Effect of different concentrations of ethrel sprayed at mature stage (165 DAFS) on endogenous ethylene in Washington Navel and cv. Lane Late sweet orange fruit.

Endogenous ethylene (µL. kg-1.hour^{-1}) 30 DAS			
Treatments	Washington Navel	Lane Late	Means (Treatment)
Control	0.59± 0.05 b	0.93±0.43 a	0.76± 0.24 b
Ethrel 250 mg L^{-1}	0.95± 0.09 a	1.18± 0.02 a	1.06± 0.16 a
Ethrel 500 mg L^{-1}	1.07±0.01 a	1.16±0.10 a	1.11±0.06 a
Ethrel 750 mg L^{-1}	1.17± 0.04 a	1.09±0.04 a	1.13± 0.06 a
Means (Cultivars)	0.94± 0.26 b	1.09± 0.11a	
LSD ($p \leq 0.05$)	Treatments=0.17, Cultivars= 0.12, Treatments × cultivars=ns		
Endogenous ethylene (µL. kg^{-1}.hour-1) 60 DAS			
Control	0.61±0.11 d	0.72± 0.27cd	0.66±0.08 c
Ethrel 250 mg L^{-1}	0.90±0.12 bc	0.97±0.11 ab	0.94±0.05 b
Ethrel 500 mg L^{-1}	0.90± 0.01 bc	1.01±0.24 ab	0.96± 0.08 b
Ethrel 750 mg L^{-1}	1.16± 0.08 a	1.09±0.19 ab	1.13± 0.04 a
Means (Cultivars)	0.89± 0.23 a	0.95± 0.16 a	
LSD ($p \leq 0.05$)	Treatments=0.14, Cultivars= ns, Treatments × cultivars=ns		
Endogenous ethylene (µL. kg-1.hour^{-1}) 90 (DAS)			
Control	0.66±0.09 e	0.75± 0.08 de	0.71±0.06 c
Ethrel 250 mg L^{-1}	0.84±0.02 cd	0.80± 0.11 cde	0.82± 0.03 b
Ethrel 500 mg L^{-1}	0.86±0.11 bcd	0.95± 0.13bc	0.90±0.06 b
Ethrel 750 mg L^{-1}	1.01± 0.11 ab	1.13± 0.15 a	1.07± 0.08 a
Means (Cultivars)	0.84±0.14 b	0.91±0.17 a	
LSD ($p \leq 0.05$)	Treatments=0.11, Cultivars= ns, Treatments × cultivars=ns		
Endogenous ethylene (µL. kg-1.hour^{-1}) 120 (DAS)			
Control	0.76± 0.05 de	0.66±0.11 e	0.71± 0.07 c
Ethrel 250 mg L^{-1}	0.84± 0.05 cd	0.81±0.08 cd	0.82± 0.02 b
Ethrel 500 mg L^{-1}	0.89± 0.06 cd	0.94 ±0.07 bc	0.91± 0.04 b
Ethrel 750 mg L^{-1}	1.05± 0.09 ab	1.19± 0.24 a	1.12±0.10 a
Means (Cultivars)	0.88±0.12 a	0.90±0.23 a	
LSD ($p \leq 0.05$)	Treatments=0.10, Cultivars= ns, Treatments × cultivars=ns		

n = 4 replications (2 fruit per replication), any two mean within a column and within a row followed by different letters are significantly different at $p \leq 0.05$ at 30, 60, 90 and 120 DAS. ns = non-significant

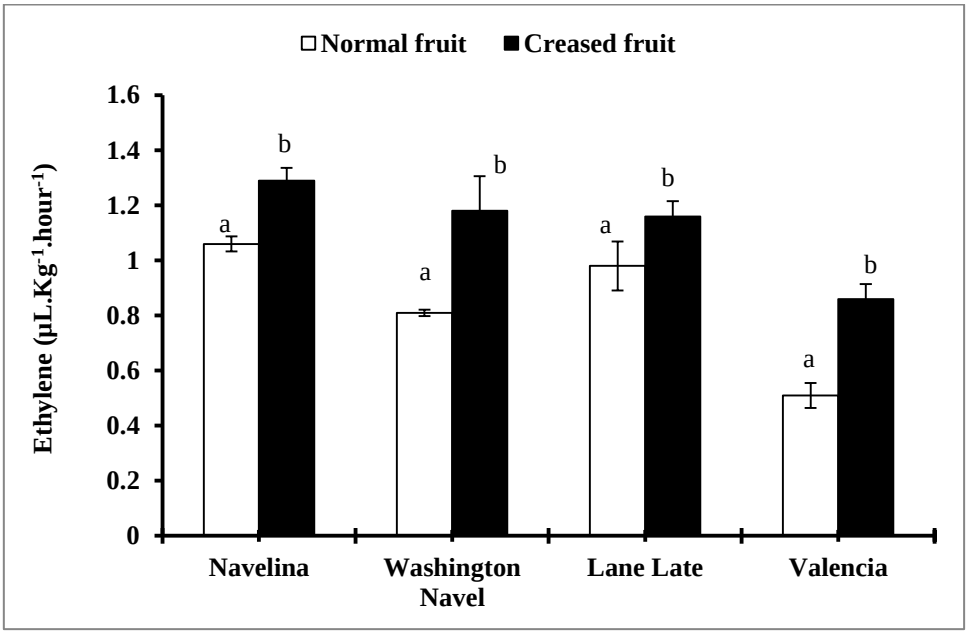

Fig 1. Comparative endogenous level of ethylene in the creased and normal fruit of different cultivars of sweet orange. n = 4 replications (2 fruit per replication). Vertical bars represent standard error means. LSD ($p \leq 0.05$), type of fruit = 0.06, cultivars = 0.08, cultivars x type of fruit = ns; ns = not-significant; Fruit types within one cultivar with diffent letters are significantly different at $p \leq 0.0$.

lowest in cv. Navelina (24.45 %) compared to cv. Washington Navel (27.06 %) and Lane Late (32.32 %) during 2011. The interaction between the treatments and the cultivars were also found to be non-significant ($p \leq 0.05$) in CI during 2011 and 2012.

Discussion

Higher concentrations of endogenous ethylene in the creased fruit than the normal ones in different cultivars of sweet orange including Navelina, Washington Navel, Lane Late and Valencia Late (Fig. 1) demonstrated that endogenous ethylene is an important factor in causation of creasing of sweet orange fruit. Increased ethylene production in the creased fruit of sweet orange may possibly be ascribed to the increased activities of 1-aminocyclopro- pane-1-carboxylase synthase (ACC synthase, ACS) and ACC oxidase in the albedo and/or flavedo tissues of the rind. The activities of these enzymes in the albedo and/or flavedo tissues of the rind in sweet orange are yet to be investigated. Earlier, Hyodo (1977) reported active ethylene biosynthesis in the albedo tissue of 'Satsuma' mandarin fruit. Increased levels of endogenous ethylene in creased albedo tissue and fruit rind has been previously reported in Valencia and Washington Navel orange by (Monselise et al., 1976; Pham, 2009) respectively. ACC is an intermediate pathway of ethylene biosynthesis in the albedo tissues. But 1-aminocyclopropane-1-carboxylic acid (ACC) formation and ACC conversion to ethylene are increased by aging and wounding in the albedo tissue of citrus fruit (Hyodo and Nishino, 1981). All the treatments of exogenous application of ethrel applied at 165 (DAFS) have significantly ($p \leq 0.05$) elevated the levels of endogenous ethylene in the fruit 30, 60, 90, 120 DAS compared to the control (Table. 1), consequently promoting creasing on the fruit in Washington Navel and Lane Late sweet orange (Fig. 2). Promotion of creasing with exogenous application of ethrel due to increased ethylene production in the fruit of both cultivars also suggested the involvement of ethylene in creasing of sweet orange fruit. Exogenous application of ethylene biosynthesis inhibitors such as AVG or $CoSO_4$ at the golf ball stage significantly reduced CI in Navelina, Washington Navel and Lane Late cultivars of sweet orange during 2011 and 2012 (Fig. 3 and 4), which further demonstrated that ethylene biosynthesis and/or its action is involved in creasing. The increased ethylene production was parallel to the increase in ACC contents, but the level of S-adenosylmethionine (SAM) was unaffected, suggesting that the conversion of SAM to ACC is a key reaction in the production of ethylene (Yu and Yang, 1980). It is well known that AVG and CoSO4 are inhibitors of ethylene biosynthesis which are involved in the conversion of SAM to ACC, eliminating the increase in ACC formation and ethylene production through the action of the ACC oxidase enzyme (Yu and Yang, 1980; Even-Chen et al., 1982; Hyodo and Nishino, 1981; Ladaniya, 2007). A substantial reduction in creasing of the fruit with the exogenous application of putrescine applied at the golf ball stage in sweet orange (data not included) also supports the involvement of ethylene in creasing. Polyamines are known to inhibit ethylene biosynthesis by inhibiting ACC synthase enzyme (Liu et al., 2006; Even and Melberg, 1989). Similarly, the pre-harvest spray application of putrescine (1-2 mM) significantly suppressed ethylene biosynthesis and consequently reduced the activities of fruit softening-related enzymes such as *exo*-PG, *endo*-PG, PE and EGase in the skin and pulp tissues of plum (*Prunus salicina* Lindl. cv. Angelino) fruit during cold storage (Khan et al., 2007). Higher endogenous levels of ethylene in the creased fruit, acceleration of creasing with exogenous applications of ethrel, and reduced CI with ethylene inhibitors indicated the involvement of ethylene in creasing of sweet orange fruit. Although, sweet orange fruit is a non-climacteric, and produces only a small amount of ethylene (<0.1 µL. $Kg^{-1}.hour^{-1}$), which induces changes in fruit colour, flavour, chemical composition and texture in citrus fruit (Aharoni, 1968; Ekas, 1970; Ladaniya, 2007). Ethylene plays a major role in regulating ripening and softening of fruit (Ayub et al., 1996; Hadfield et al., 2000), and accordingly, the expression of some ripening-related and cell wall-associated genes and activities, including those of PGs (Hiwasa et al., 2003; Sitrit and Bennett, 1998), expansins (Rose et al., 1997), and EGases (Lashbrook et al., 1994). Possibly higher levels of ethylene in the fruit may have induced architectural changes in structures of cell wall components in the albedo tissue including activities of various enzymes involved in cell wall degradation. Similarly, higher activities of PE, *exo*-PG, *endo*-PG, and EGase in the albedo and flavedo tissues of creased fruit than normal ones have been reported to be associated with greater losses of pectins and starch in albedo cell walls , consequently leading to creasing (Li et al., 2009; Saleem et al., 2014). In conclusion, higher levels of endogenous ethylene in the creased fruit than the normal ones in Navelina, Washington Navel, Lane Late and Valencia Late cultivars of sweet orange, acceleration of CI with exogenous application of ethrel, and its reduction with ethylene biosynthesis inhibitors indicate the involvement of ethylene in creasing of sweet orange fruit.

Materials and Methods

Plant materials

Four different experiments were conducted in a commercial orchard located at Gingin (latitude 31° 21′ South, longitude 155° 55′ East), Western Australia. Twenty five-year old uniform sweet orange tree on trifoliate orange (*Poncirus trifoliate* Raf.) rootstock, planted at 2.7 x 7.5 m spacing on a north-south orientation were used in the experiments. These experiments were conducted on Navelina, Washington Navel, Lane Late and Valencia cultivars of sweet oranges. The orchard soil was sandy loam. All cultural practices, other than the experimental treatments, were similar to those practiced in commercial orchards (Mould and Tugwell, 1999).

Determination of comparative endogenous levels of ethylene in the normal and creased fruit of different cultivars of sweet orange

The experiment was conducted to determine the comparative endogenous levels of ethylene in the normal and the creased fruit of Navelina, Washington Navel, Lane Late and Valencia Late sweet orange. The experimental arrangement was randomised complete block design with two-factor factorial (cultivars and type of fruit) with a single tree as an experimental unit and four replicates. Two-fruit of each category (normal and creased) per tree were harvested at harvest maturity and endogenous levels of ethylene were determined.

Effects of exogenous application of ethrel on endogenous levels of ethylene and incidence of creasing in Washington Navel and Lane Late sweet orange

An aqueous solution containing different concentrations (250, 500 and 750 mg L^{-1}) of Ethrel® [2-chloroethylphosphonic

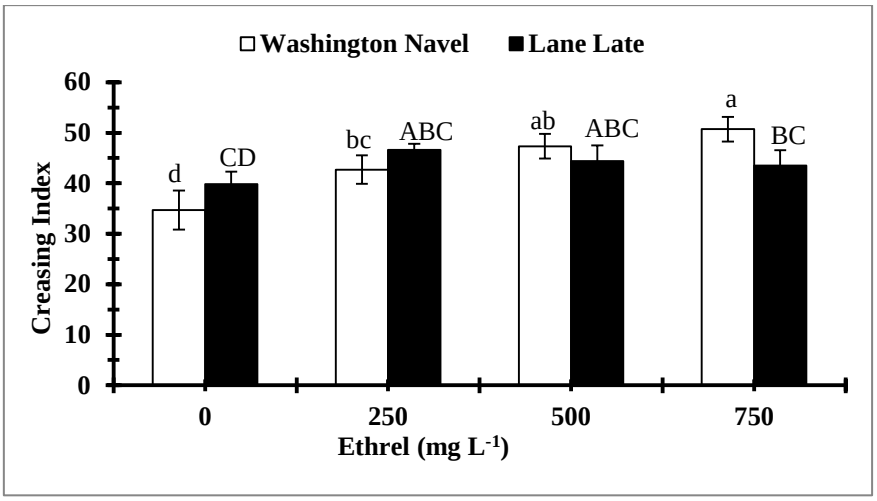

Fig 2. Effect of exogenous application of different concentrations of ethrel on CI at mature fruit in Washington Navel and Lane Late fruit. n = 3 replications (35 fruit per replication). Vertical bars represent S.E. of means. LSD ($p \leq 0.05$) for creasing index, Treatments = 4.34, Cultivars = ns, Treatments × cultivars = 6.14; ns = not-significant; Any two mean with different lower case letters represent significant differences at $p \leq 0.05$ in cultivar Washington Navel and upper case letters for cultivar Lane Late.

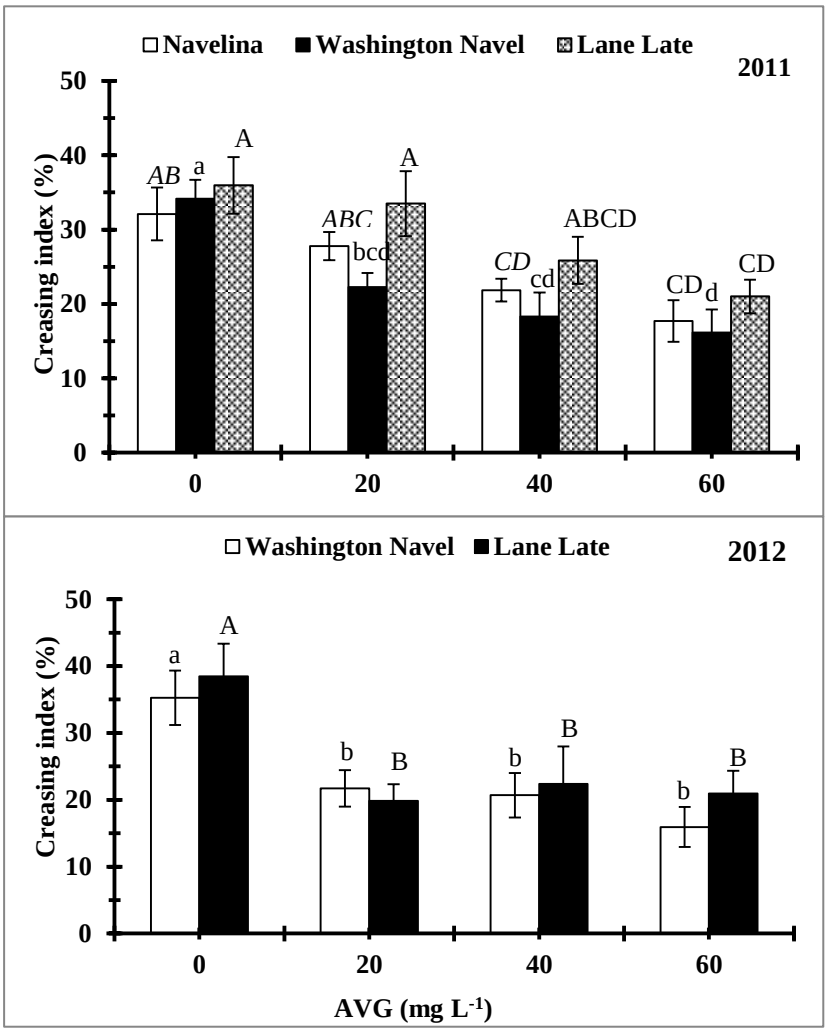

Fig 3. Effect of exogenous application of different concentrations of aminoethoxyvinylglycine (AVG) applied at golf ball stage on CI in different cultivars of sweet orange. n = 4 replications (35 fruit per replication). Vertical bars represent S.E. of means. LSD ($p \leq 0.05$) for 2011, Treatments = 5.32, Cultivars = 4.61, Treatments × cultivars = ns, LSD ($p \leq 0.05$) for 2012, Treatments = 8.08, Cultivars = ns, Treatments × cultivars = ns; ns = not-significant. Any two mean with different lower case letters represent significant differences at $p \leq 0.05$ in cultivar Washington Navel, upper case letters for cultivar Lane Late and italic upper case letters for Navelina.

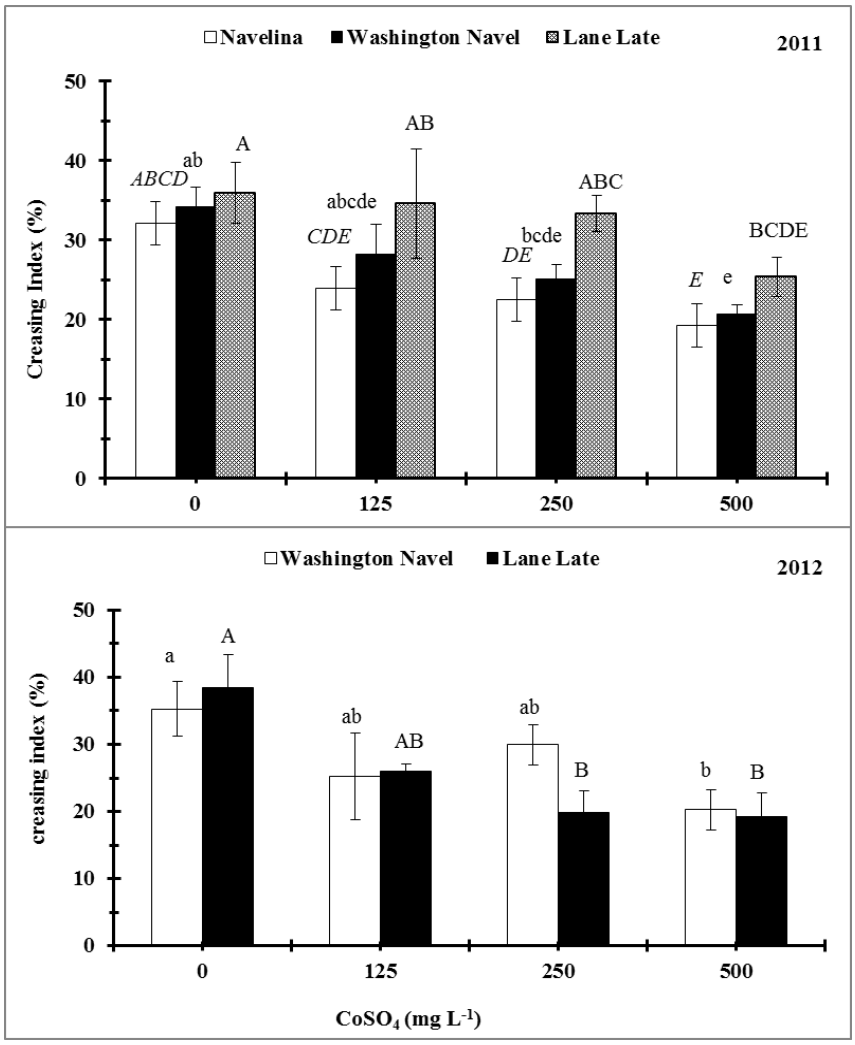

Fig 4. Effect of exogenous application of different concentrations of cobalt sulphate ($CoSO_4$) applied at golf ball stage on CI in different cultivars of sweet orange. n = 4 replications (35 fruit per replication). Vertical bars represent S.E. of means. LSD ($p \leq 0.05$) for 2011, Treatments = 515, Cultivars = 4.46, Treatments × cultivars = ns, LSD ($p \leq 0.05$) for 2012, Treatments = 8.55, Cultivars = ns, Treatments × cultivars = ns; ns = not-significant. Any two mean with different lower case letters represent significant differences at $p \leq 0.05$ in cultivar Washington Navel, upper case letters for cultivar Lane Late and italic upper case letters for Navelina.

acid (Rhone-Poulenc Rural Australia Pty Ltd, Baulkham Hills, NSW, Australia)] and 0.05% 'Tween 20' as a surfactant was sprayed onto the whole tree till run off at fruit maturity (01 April, 2012: 165 Days after fruit set) in cv. Washington Navel and Lane Late using a sprayer (The Selecta Trolleypak Mk II, Acacia Ridge, Australia). Control trees were kept as unsprayed. The experimental lay out was randomised complete block design with two-factor factorial (treatments and cultivars). A single tree was considered as an experimental unit and included four replications. At commercial harvest, 35 ripe fruit per tree were randomly harvested around the tree canopy. The endogenous levels of ethylene in the fruit were determined 30, 60, 90 and 120 DAFS. The creasing indexes in both cultivars were determined at fruit harvest.

Effect of exogenous application of ethylene inhibitor (AVG) on the incidence of creasing in different cultivars of sweet orange in 2011 and 2012

The aim of this experiment was to down regulate the ethylene production using AVG, which is an inhibitor of ethylene biosynthesis and consequently shows its inhibition effects on creasing. An aqueous solution containing different concentrations (20, 40, 60 mg L^{-1}) of AVG (as ReTain® from Valent BioSciences®, Chatswood, NSW, Australia) and 'Tween 20' (0.05 %) as a surfactant was sprayed at the golf ball stage (fruit diameter 40±5 mm) onto the whole trees of Navelina, Washington Navel, Lane Late sweet oranges in 2011 and Washington Navel, Lane Late in 2012 using a sprayer (The Selecta Trolleypak Mk II, Acacia Ridge, Australia). Untreated trees were served as a control. The experiment was laid out by following two factors (AVG treatments and cultivars) factorial randomised block design with four replicates. Single tree was treated as an experimental unit. At ripe stage, 35-fruit per tree were randomly harvested around the tree canopy. The incidence of creasing was recorded and expressed as a creasing index (CI, %).

Effects of exogenous application of ethylene inhibitor ($CoSO_4$) on the incidence of creasing in different cultivars of sweet orange

In this experiment, an aqueous solution containing different concentrations (125, 250 and 500 mg L^{-1}) of CoSO4 and

'Tween 20' (0.05 %) as a surfactant was sprayed at golf ball stage onto the whole trees of Navelina, Washington Navel and Lane Late sweet oranges in 2011 and Washington Navel, Lane Late in 2012 using the same sprayer as mentioned in the experiment 4. The experiment used a completely randomized design with two factors including $CoSO_4$ treatments and cultivars. Single tree was treated as an experimental unit and the experiments included four replications. At ripe stage, 35-fruit per tree were randomly harvested around the tree canopy. Creasing index was recorded from these harvested fruits.

Determination of endogenous ethylene

The endogenous level of ethylene was determined by following the method described earlier by Pranamornkith et al. (2012). After harvesting fruit were transported to the laboratory within three to four hours. The endogenous levels of ethylene were determined using an ETD 300 ethylene detector (Sensor sense B.V, Nijmegen, The Netherlands). The fruit were washed to remove all the dust. Fruit free from diseases were used for determination of ethylene. Each fruit sample was weighed before transferring into the cuvettes i.e. in a 1.0 L air-tight jar, fitted with two outlets. All the cuvettes were sealed tight to prevent leakage. Before connecting flow to the cuvette, ensured that the output of the cuvette is not blocked, in order to avoid pressure built up in the cuvette. Each sample was run for 20 minutes and flow rate was 4.0 L $hour^{-1}$. The ethylene was expressed in ($\mu L.Kg^{-1}.hour^{-1}$).

Creasing index (%)

CI was determined using 4-point hedonic scale based on the symptoms of creasing on the surface of individual fruit. The scale used was: 0 = no creasing; 1= slightly creased (1 to 25% fruit surface with symptoms); 2= moderately creased (26 to 50% fruit surface with symptoms); 3= severely creased (> 51% fruit surface with symptoms). Creasing index (CI) percent was calculated as described by Treeby et al. (1995) using the formula;

$$\text{Creasing index \%} = \frac{[\sum (\text{Creasing category x number of fruit in rating category})] \text{ x } 100}{\text{Highest rating value x Total number of fruit assessed}}$$

Statistical analysis

The experimental data were subjected to two-way analysis of variance (ANOVA) depending upon experimental design, using GenStat 14th edition (Lawes Agricultural Trust, Rothamsted experimental station, U.K). The effects of treatments, cultivars and their interactions on different parameters were assessed within ANOVA. The least significant differences (LSD) were calculated following a significant Duncan's test at $p \leq 0.05$. All the assumptions of analysis were checked to ensure validity of statistical analysis.

Conclusion

Higher levels of endogenous ethylene in the creased fruit than the normal ones in Navelina, Washington Navel, Lane Late and Valencia Late cultivars of sweet orange, acceleration of CI with exogenous application of ethrel, and its reduction with ethylene biosynthesis inhibitors indicated the involvement of ethylene in creasing of sweet orange fruit.

Acknowledgements

We thank Professor Fallahi, University of Idaho, Parma Research and Extension Center, USA for his suggestions and critically reviewing the manuscript. Zahoor Hussain is grateful the Department of Environment and Agriculture, Curtin University, Perth, and the University of Sargodha for providing financial assistance under Faculty Development Program and study leave. He is also thankful to Ms Susan Petersen and colleagues for their assistance in the laboratory and field work. We thank Mr. J Marten, Farm Manager, Westralian Fruits Pty Ltd, Gingin, Western Australia for providing us access to the orchard, trees and fruit for this research.

References

Agustí M, Martinerz-Fuentes A, Mesejo C (2002) Citrus fruit quality physiological basis and techniques of improvement. Agrociencia. 6: 1-16.

Agustí M, Almela V, Juan M, Mesejo C, Martinez-Fuentes A (2003) Rootstock influence on the incidence of rind breakdown in 'Navelate' sweet orange. J Hortic Sci Biotechnol.78: 554-558.

Agustí M, Almela V, Juan M, Martinez-Fuentes A, Mesejo C (2004) Quality of citrus for fresh consumption as affected by water relationships. Acta Hortic. 632: 141-148.

Aharoni Y (1968) Respiration of oranges and grape fruit harvested at different stages of development. Plant Physiol. 43: 99-102.

Ali A, Summers LL, Klein GJ, Lovatt CJ (2000) Albedo breakdown in California. Proc Int Soci Citri. 2: 1090-1093.

Al-Mughrabi MA, Bacha MA, Abdel-Rahman AO (1989) Influence of pre-harvest application of ethrel and 2, 4-D on fruit quality of 'Baladi' oranges. J King Saud Univ Agric Sci. 1: 95-102.

Ayub R, Guis M, Amor MB, Gillot L, Roustan JP, Latche A, Bouzayen M, Peach JC (1996) Expression of ACC oxidase antisense gene inhibits ripening of cantaloupe melon fruits. Nat Biotechnol.14:862-866.

Bleecker AB (2000) Ethylene: A gaseous signal molecule in plants. Ann Rev Cell Dev Biol. 16: 1-18.

Bower JP (2000) Prediction and physiology of creasing. Proc Int Soci Citri: 1089.

Bower JP (2004) The physiological control of citrus creasing. Acta Hortic. 632: 111-115.

Burg SP (2004) Heat transfer and water loss, p. 241-301. In: Postharvest physiology and hypobaric storage of fresh produce. CABI Publishing. p. 654.

Ekas IL (1970) Respiratory response, ethylene production, and response to ethylene of citrus fruit during ontogeny. Plant Physiol. 45: 334-338.

Even-Chen Z, Mattoo AK, Goren R (1982) Inhibition of ethylene biosynthesis by aminoethoxyvinylglycine and by polyamines shunts label from 3, 4-[^{14}C] Methionine into Spermidine in aged orange peel discs. Plant Physiol. 69:385-388.

Evans PT, Malmberg RL (1989) Do polyamines have roles in plant development? Ann Rev Plant Physiol Plant Mol Bio. 40: 235-269.

Gambetta G, Arbiza H, Ferenczi A, Gravina A, Orlando L, Severino V, Telias A (2000) Creasing in 'Washington Navel' orange in Uruguay: study and control. Proc Int Soci Citri IX Congress, Florida, USA. p. 453-455.

Gilfillan I, Stevenson J, Wahl J, Holmden E (1981) Control of creasing in navels with gibberellic acid. Proc Int Soci Citri, Tokyo, Japan. p. 224-226.

Hadfield KA, Dang T, Guis M, Pech JC, Bouzayen M, Bennet AB (2000) Characterization of ripening regulated cDNAs and their expression in ethylene suppressed Charentais melon fruit. Plant Phsyiol. 122: 977-983.

Hiwasa K, Kinugasa Y, Amano S, Hashimoto A, Nakano R, Inaba A, Kubo Y (2003) Ethyelen is required for both the initation and preogression of softening in pear (*Pyrus communis* L.) fruit. J Exp Bot. 54: 771-779.

Holtzhausen LC (1981) Creasing: formulating a hypothesis. Proc Intl Soc Citri. 1:201-204.

Hyodo H (1977) Ethylene production by albedo tissue of Satsuma mandarin (*Citrus unshiu* Marc.) fruit. Plant Physiol. 59: 111-113.

Hyodo H, Nishino T (1981) Wound-induced ethylene formation in albedo tissue of citrus fruit. Plant Physiol. 67: 421-423.

Jones WW, Embleton TW, Garber MJ, Cree CB (1967) Creasing of orange fruit. Hilgardia. 38: 231-244.

Khan AS, Singh Z, Abbasi NA (2007) Pre-storage putrescine application suppresses ethylene biosynthesis and retards fruit softening during low temperature storage in 'Angelino' plum. Postharvest Biol Technol. 47:36-46.

Ladaniya M (2007) Citrus Fruit: Biology, Technology and Evaluation. Academic Press. p: 558.

Lashbrook CC, Gonzalez-Bosch C, Bennett AB (1994) Two divergent endo-β-1-4,-glucanase gene exhibit overlapping expression in ripening fruit and abscising flowers. Plant Cell. 10: 1485-1493.

Li J, Zhanga P, Chena J, Yao Q, Jiang Y (2009) Cellular wall metabolism in citrus fruit pericarp and its relation to creasing fruit rate. Scientia Hort. 122: 45-50.

Liu Ji-Hong, Honda C, Moriguch TM (2006) Involvement of polyamine in floral and fruit development. JARQ. 40(1): 51-58.

Manjunatha G, Gupta KJ, Lokesh V, Mur LAJ, Neelwarne B (2012). Nitric oxide counters ethylene effects on ripening fruits. Plant Signal Behav. 74: 476-483.

Monselise SP, Weiser M, Shafir N, Goren R, Goldschmidt EE (1976) Creasing of orange peel-physiology and control. J Hort Sci. 51: 341-351.

Moulds G, Tugwell B (1999) Citrus growing manual. A manual of quality decision making. Horticultural Research Development Corporation.

Pham TTM (2009) Pre-harvest factors affecting fruit quality in sweet oranges with an emphasis on albedo breakdown. Ph.D thesis Curtin University Perth- Australia.

Pham TTM, Singh Z, Behhoudian H (2012) Different surfactants improve calcium uptake into leaf and fruit of 'Washington Navel' sweet orange and reduced albedo breakdown. J Plant Nutrition. 35: 889-904.

Pranamornkith T, East A, Heyes J (2012) Influence of exogenous ethylene during refigerated storage on storability and quality of *Actinidia chinensis* cv. Hort 16 A. Postharvest Biol Technol. 64:1-8.

Rath AC, Prentice AJ (2004) Yield increase and higher flesh firmness of ' Arctic Snow' nectarines both at harvest in australia and after export to Taiwan follwing pre-harvest application of ReTain® plant growth regulator (Aminoethoxylycine, AVG). A J Exp Agr. 44:343-351.

Rose JKC, Lee HH, Bennett AB (1997) Expression of a divergent expansin gene is fruit specific and ripening-regulated. Proc Natl Acad Sci, USA. 94: 5955-5960.

Saleem BA, Hassan I, Singh Z, Malik AU, Pervez MA (2014) Comparative changes in the rheological properties and cell wall metabolism in rind of healthy and creased fruit of Washington Navel and Navelina sweet orange (*Citrus sinensis* [L.] Osbeck). Aust J Crop Sci. 8(1):62-70.

Saltveit ME (1999) Effect of ethylene on quality of fresh fruits and vegetables. Postharvest Biol Technol. 15:279-292.

Sitrit Y, Bennett AB (1998) Regulation of tomato fruit polygalacturonase mRNA accumulation by ethylene: a reexamination. Plant Physiol. 129: 1755-1762.

Treeby MT, Milne DJ, Storey R, Bevington KB, Loveys BR, Hutton R (2000) Creasing in Australia: causes and control. Proc Intl Soc Citri IX Congress, Florida, USA. p. 1099-1103.

Treeby MT, Storey RA, Bevington KB (1995) Rootstock, seasonal, and fruit size influences on the incidence and severity of albedo breakdown in 'Bellamy' Navel oranges. Aust J Exp Agr. 35:103-108.

Yu Yeong-biau, Yang SF (1980) Biosynthesis of wound ethylene. Plant Physiol. 281-285.